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# Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance

Richard L. Fery*, Philip D. Dukes Sr.

*US Vegetable Laboratory, Agricultural Research Service, US Department of Agriculture, 2875 Savannah Highway, Charleston, SC 2941-5334, USA*

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**Abstract**

Replicated field studies were conducted over a three-year period to assess the yield-reducing potential of southern blight (*Sclerotium rolfsii*) on cowpea, and to evaluate the range of genetic variability in cowpea germplasm for resistance. Southern blight was shown to have a considerably greater impact on dry-seed yields than that suggested by a review of the published literature. The disease caused a dry-seed yield loss of 53.4% in one study, and losses approaching 50% were observed for some cultivars in a second study. Reduced pod numbers likely accounted for most of the reduced dry-seed yields. There is significant variability in cowpea germplasm for resistance to southern blight. Two cultivars, Brown Crowder and Carolina Cream, exhibited promising levels of resistance in two years of testing. © 2002 Published by Elsevier Science Ltd.

*Keywords:* Cowpea; *Vigna unguiculata*; Southern blight; Stem rot; *Sclerotium rolfsii*; Disease resistance

Southern blight, a stem disease incited by *Sclerotium rolfsii* Sacc., is recognized as a common disease of cowpea (*Vigna unguiculata* (L.) Walp.) worldwide. Aycock (1966) described the general effects of *S. rolfsii* in inciting southern blight on numerous species of host plants. Our observation of susceptible cowpea plants indicates that infection initially results in decay of the stem in the top 2 cm of the soil. The first observed symptoms are usually a general wilting and yellowing of plants, which is soon followed by drying of foliage and plant death. The stems of plants with advanced disease development characteristically exhibit tan to brown sclerotial bodies and white mycelial growth on the epidermis of the stem at the soil surface (Karat et al., 1985). Punja (1985) observed that most efforts to control *S. rolfsii* have met with limited success, and he speculated that this is due in part to the pathogen's extensive host range, prolific growth, and ability to produce large numbers of sclerotia that may persist in the soil for many years.

Although southern blight has long been considered an important disease of cowpea, there is only limited information in the scientific literature that deals with either resistance to the disease in cowpea or yield losses in cowpea attributable to the disease. Aycock (1966) cited several published studies, as early as, 1926 reporting that cowpea is a host of *S. rolfsii*, and several researchers (Hoffmaster et al., 1943; Epps et al., 1951; Jenkins and Averre, 1986) have reported that southern blight is an important disease of cowpea. Toler et al. (1963) assayed the economic importance of various diseases of cowpea in Georgia, and they concluded that southern blight was a widespread problem but was responsible for an economic loss of $<1\%$. Muqit et al. (1996) evaluated 20 cowpea lines for reaction to *S. rolfsii*, and they reported that 'BARI Felon-1' was moderately susceptible to the pathogen and the remainder of the tested lines were susceptible. Karat et al. (1985) evaluated eight cowpea cultivars for reaction to *S. rolfsii* in a replicated field trial, and observed that southern blight symptoms were quite severe on 'C-152' and virtually nonexistent on 'NP-3'. Nwakpa and Ikotun (1988) screened 20 cowpea cultivars for their reaction to *S. rolfsii*. They concluded that none of the cultivars were immune to the disease, but noted that the plant reactions ranged from resistant to highly susceptible.

Many researchers use the frequency of plant mortality to estimate the impact of southern blight on crop yields.

*Corresponding author. Tel.: +1-843-556-0840; fax: +1-843-763-7013.

*E-mail address:* rfery@awod.com (R.L. Fery).

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However, our observations of this disease on cowpea suggest that the impact of southern blight on cowpea yield may be more attributable to reduced plant vigor than to plant mortality per se. Additionally, our observations of an inoculated, unreplicated field evaluation of 200 cultivars in their reaction to *S. rolfsii* suggested that there might be considerable variability in cowpea germplasm for resistance to the pathogen (R.L. Fery and P.D. Dukes Sr., unpublished data). The objectives of the studies reported here are to assess the yield-reducing potential of the disease on cowpea and to evaluate the range of genetic variability in cowpea for resistance.

## 1. Materials and methods

The data reported here are from replicated field studies conducted at the US Vegetable Laboratory, Charleston, SC, during the years 1984, 1985, and 1986. Seeds of all cowpea accessions evaluated in these studies were produced by the authors prior to the initiation of specific studies and all seed lots exhibited high germination. The severity of southern blight symptoms in all of the studies was assessed by assigning each plant a subjective score for severity of wilting. Each plant in the 1984 and 1986 studies was assigned a second subjective score based on the severity of stem-lesion development. The following scale was used to score for severity of wilting: 1—no wilting symptoms, 2—slight or partial wilting, 3—general plant wilting, 4—permanent wilt, and 5—dead plant. The following scale was used to score for severity of stem lesions: 1—no stem lesion, 2—small stem lesion (<25% of the stem circumference), 3—moderate stem lesion (26–50% of the stem circumference), 4—large stem lesion (>51% of the stem circumference), and 5—dead plant (stem completely girdled).

### 1.1. Inoculum preparation and inoculation procedures

The isolate of *S. rolfsii* utilized in these studies was collected at Charleston, SC, from a field-grown pepper plant using locally prepared, acid potato dextrose agar (APDA) medium. The isolate was subsequently maintained as dry sclerotia stored in glass vials at room temperature. The inoculum for field experiments was prepared using a four-step procedure. First, dry sclerotia were surface sterilized (dipped in 95% ethanol and flamed) and placed in the center of APDA plates, and the plates were incubated at 30°C until the resulting cultures produced new sclerotia. Second, 9 mm diameter plugs of APDA media containing both sclerotia and mycelium were used to infest 2.8 l Fernbach flasks (10 plugs/flask) containing an autoclaved mixture of 1000 g clean sharp sand (air dry), 200 g yellow corn meal, and 300 ml of distilled water. Third, the infested corn meal-sand media in the Fernbach flasks were incubated at 30°C for 20 days (flasks shaken daily for the first seven days). Fourth, the corn meal-sand media containing high concentrations of newly developed, mature sclerotia were removed from the Fernbach flasks and mixed with sufficient quantities of additional clean sharp sand to produce inoculum with the desired concentration of sclerotia. Individual plants were prepared for inoculation by removing all top soil within ≈5 cm of the stem to a depth of ≈2 cm. A measuring spoon was then used to place one tablespoon of inoculum containing ≈20 sclerotia in direct contact with the entire circumference of the exposed stem. Finally, the inoculum in the 'dished out' area around each stem was lightly covered with top soil.

### 1.2. Relationship between time of inoculation and dry-seed yield (1984 study)

This field study was conducted to assess the relationship between the time of inoculation of the cowpea cultivar Pinkeye Purple Hull with *S. rolfsii* sclerotia and seed yield. Pinkeye Purple Hull is a horticultural-type cultivar that is representative of the predominant cultivar class grown as a vegetable crop in the southern United States for processing. Our observations over a number of years suggested that this cultivar is moderately susceptible to southern blight.

The design of the experiment was a randomized complete block with six replications. Each replicate included the following treatments: control (not inoculated), early inoculation (inoculated 35 days after planting with 20 sclerotia/plant), medium inoculation (inoculated 45 days after planting with 20 sclerotia/plant), and late inoculation (inoculated 55 days after planting with 20 sclerotia/plant). Each plot consisted of a single row of 10 plants spaced 90 cm apart; the rows were centered on beds spaced 1 m apart. The plots were planted on 15 June 1984 by dropping three seeds/hill, and each hill was thinned to a single plant immediately after the appearance of the first true leaves.

Each plant was scored for severity of wilting on 24 August 1984. Each plot was harvested when an estimated 95% of its pods had dried (29 August 1984–7 September 1984). The pods were placed in a forced-air seed drier (35°C) immediately after harvest, shelled after removal from the drier, and the seeds were stored in a seed-storage facility (50% RH and 10°C) for several weeks before weighing. Immediately after the harvesting operation was completed, the root of each plant was lifted from the soil and the stem was evaluated for severity of stem-lesion development. The wilt index, stem-lesion index, and dry seed yield/plant data were analyzed by using Analysis of Variance procedures and

the means were separated by using Duncan's multiple range test.

### 1.3. Evaluation of a collection of cowpea cultigens (1985 study)

This study was conducted to assess the yield-reducing potential of southern blight on cowpea yields, to characterize the effect of the disease on the various yield components, and to evaluate the range of genetic variability available in cowpea germplasm for resistance to the disease. The experimental design was a split-plot with six replications. Each replicate included a two-row plot for each of the following 12 cowpea cultigens: Pinkeye Purple Hull, Brown Crowder, Carolina Cream, Colossus-80, PI 354648, Speckled Purple Hull, Mississippi Purple, Climax, Magnolia Blackeye, Mississippi Silver, Mississippi Cream, and New Era. Based on observations of earlier field plantings, these cultigens were selected to represent a range of reactions in cowpea to the southern blight pathogen. Each plot was split into two subplots of one row each. Each subplot consisted of a single row of 20 plants spaced 90 cm apart; the single-row subplots were centered on beds spaced 1 m apart. One of the two subplots within each plot was randomly assigned as the inoculated subplot; the remaining subplot served as the uninoculated control.

The plots were planted on 4 June 1985 by dropping three seeds/hill, and each hill was thinned to a single plant immediately after the appearance of the first true leaves. The inoculated subplots were inoculated with 20 sclerotia, 31 days after planting. Each plant was scored for severity of wilting on 12 August 1985. Each subplot was harvested when an estimated 95% of its pods had dried (13 August 1985–6 September 1985). The pods were counted, dried in a forced-air drier as described above, and shelled. Seed yields were sub-sampled in order to determine the average weight/100 seeds. Number of seeds/pod was determined as (weight of seed/weight/seed)/number of pods. Analysis of Variance procedures were utilized to determine the significance of main effects and interactions effects, and the significance level between inoculation treatments within the same cowpea genotype was determined by calculation of LSDs for interaction effects.

### 1.4. Re-evaluation of selected cowpea cultigens (1986 study)

This study was conducted to confirm the basic results of the cowpea germplasm study conducted in 1985. The experimental design, methodology, and methods of data analysis were identical to the design, methodology, and methods of data analysis of the six-replicate, split-plot design used for the 1985 study except that fewer cowpea accessions were evaluated and data were collected on both wilting and stem-lesion development. Each replicate included two-row plots of Brown Crowder, Carolina Cream, and Magnolia Blackeye. Brown Crowder and Carolina Cream exhibited high levels of resistance to *S. rolfsii* in the 1985 study and Magnolia Blackeye was extremely susceptible to the pathogen. The plots were planted on 30 May 1986 and the inoculated subplot treatments were inoculated with 20 sclerotia, 31 days after planting. Each plant was scored for severity of wilting on 7 August 1986. The Magnolia Blackeye and Carolina Cream subplots were harvested on 5 August 1986 and the Brown Crowder subplots were harvested during 12–15 August 1986. The roots of all plants were lifted from the soil on 11 September 1986, and each stem was scored for the presence of stem lesions.

## 2. Results and discussion

### 2.1. Relationship between time of inoculation and dry-seed yield (1984 study)

An examination of the results of the early, medium, and late sclerotia inoculation treatments of the cultivar Pinkeye Purple Hull indicates that early inoculation with 20 sclerotia/plant, 35 days after planting, was the most effective timing for inoculation of cowpea plots (Table 1). This treatment caused a 53.4% reduction in seed yield from the uninoculated control, and an examination of the stem-lesion data suggests that most of the plants developed large stem lesions prior to harvest. The medium inoculation treatment at 45 days after planting also resulted in the development of large

Table 1
Effect of time of inoculation with *Sclerotium rolfsii* sclerotia on the development of southern blight symptoms and total dry seed yield in field-grown 'Pinkeye Purple Hull' cowpea (1984 study)

| Time of inoculation[a] | Wilt index[b] | Stem-lesion index[c] | Dry seed yield/plant (g) |
|---|---|---|---|
| Control | 1.13 b[d] | 1.52 c | 61.8 a |
| Early | 1.76 a | 4.00 a | 28.8 c |
| Medium | 1.75 a | 3.48 a | 47.2 b |
| Late | 1.07 b | 2.71 b | 62.8 a |

[a] Inoculation treatments: control, not inoculated; early, inoculated 35 d after planting; medium, inoculated 45 d after planting; and late, inoculated 55 d after planting (inoculum level: 20 sclerotia/plant).

[b] Each plant was rated on a scale of 1–5; 1—no wilting symptoms, 2—slight or partial wilting, 3—general plant wilting, 4—permanent wilt, and 5—dead plant.

[c] Each plant was rated on a scale of 1–5; 1—no stem lesion, 2—small stem lesion ($\leqslant$25% of the stem circumference), 3—moderate stem lesion (26–50% of the stem circumference); 4—large stem lesion ($\geqslant$51% of the stem circumference) and 5—dead plant (stem completely girdled).

[d] Mean separation within columns by Duncan's multiple range test, $P \leqslant 0.05$.

Table 2
Influence of southern blight on number of pods/plant, number of seeds/pod, weight/100 seeds, dry seed yield/plant, and wilt index of susceptible and resistant cowpea accessions (1985 study)

| Accession/inoculation treatment | Pods/plant (no.) | Seeds/pod (no.) | Wt. /100 dry seeds (g) | Dry-seed yield (g/plant) | Wilt index[a] |
|---|---|---|---|---|---|
| *Pinkeye Purple Hull* | | | | | |
| Uninoculated | 34.6 | 10.7 | 13.2 | 49.0 | 1.1 |
| Inoculated[b] | 30.7 | 10.7 | 12.8 | 42.3 | 1.3 |
| Significance[c] | NS | NS | NS | NS | NS |
| *Brown Crowder* | | | | | |
| Uninoculated | 41.3 | 12.9 | 16.6 | 89.5 | 1.0 |
| Inoculated | 37.3 | 13.1 | 16.4 | 80.3 | 1.1 |
| Significance | NS | NS | NS | NS | NS |
| *Carolina Cream* | | | | | |
| Uninoculated | 30.7 | 11.2 | 10.8 | 40.7 | 1.1 |
| Inoculated | 27.2 | 11.3 | 10.7 | 36.0 | 1.1 |
| Significance | NS | NS | NS | NS | NS |
| *Colossus 80* | | | | | |
| Uninoculated | 21.4 | 11.8 | 25.6 | 65.0 | 1.1 |
| Inoculated | 16.8 | 11.0 | 26.3 | 48.7 | 1.7 |
| Significance | NS | * | * | * | * |
| *PI 354648* | | | | | |
| Uninoculated | 44.5 | 14.3 | 11.6 | 73.7 | 1.2 |
| Inoculated | 34.0 | 13.9 | 11.3 | 54.3 | 2.3 |
| Significance | * | NS | NS | ** | *** |
| *Speckled Purple Hull* | | | | | |
| Uninoculated | 46.8 | 13.0 | 15.0 | 92.4 | 1.1 |
| Inoculated | 40.2 | 12.8 | 15.1 | 79.4 | 1.5 |
| Significance | NS | NS | NS | NS | NS |
| *Mississippi Purple* | | | | | |
| Uninoculated | 31.0 | 13.4 | 19.6 | 82.2 | 1.0 |
| Inoculated | 29.0 | 13.0 | 19.6 | 74.3 | 1.6 |
| Significance | NS | NS | NS | NS | NS |
| *Climax* | | | | | |
| Uninoculated | 54.3 | 14.7 | 14.2 | 110.8 | 1.1 |
| Inoculated | 46.8 | 13.7 | 14.0 | 92.2 | 1.5 |
| Significance | NS | * | NS | ** | NS |
| *Magnolia Blackeye* | | | | | |
| Uninoculated | 39.2 | 10.6 | 13.8 | 57.7 | 1.3 |
| Inoculated | 21.3 | 9.8 | 13.7 | 29.2 | 2.5 |
| Significance | *** | * | NS | *** | *** |
| *Mississippi Silver* | | | | | |
| Uninoculated | 33.0 | 13.7 | 19.9 | 90.8 | 1.0 |
| Inoculated | 25.0 | 13.6 | 19.0 | 65.7 | 1.7 |
| Significance | NS | NS | * | *** | ** |
| *Mississippi Cream* | | | | | |
| Uninoculated | 60.3 | 12.6 | 11.4 | 84.0 | 1.1 |
| Inoculated | 43.5 | 11.3 | 12.0 | 60.2 | 1.6 |
| Significance | *** | ** | NS | *** | * |
| *New Era* | | | | | |
| Uninoculated | 82.5 | 15.1 | 10.3 | 131.3 | 1.6 |
| Inoculated | 43.0 | 15.9 | 10.0 | 70.2 | 2.5 |
| Significance | *** | * | NS | *** | ** |

Table 2 (*continued*)

| Accession/inoculation treatment | Pods/plant (no.) | Seeds/pod (no.) | Wt. /100 dry seeds (g) | Dry-seed yield (g/plant) | Wilt index[a] |
|---|---|---|---|---|---|
| | *F ratio values for main effects and interaction effects* | | | | |
| Accession (ACC) | 9.21*** | 32.07*** | 257.12*** | 6.88*** | 5.06*** |
| Inoculation (INOC) | 76.11*** | 6.94* | 0.75NS | 97.93*** | 59.07*** |
| INOC × ACC | 6.43*** | 2.09* | 1.58NS | 4.96*** | 2.09* |

[a] Each plant was rated on a scale of 1–5; 1—no wilting symptoms, 2—slight or partial wilting, 3—general plant wilting, 4—permanent wilt, and 5—dead plant.

[b] Each plant in the inoculated plots was inoculated 31 d after seeding (inoculum level: 20 sclerotia/plant).

[c] Significance level between the inoculation treatments within the same cultivar treatment determined by calculation of LSDs for interaction effects. NS—Nonsignificant.

*Significant at $P \leqslant 0.05$.

**Significant at $P \leqslant 0.01$.

***Significant at $P \leqslant 0.001$.

stem lesions on most plants, but the impact on seed yields was considerably less (23.6% yield reduction). The late inoculation treatment 55 days after planting was implemented well after the initiation of pod set. Although the late treatment did cause a significant increase in stem lesion development, it did not adversely impact dry seed yields. Examination of the wilt data indicates that few of the inoculated plants in this study exhibited severe wilting and plant death, the classical symptoms of southern blight.

### 2.2. Evaluation of a collection of cowpea cultigens (1985 study)

Virtually, all the main effects for accession and inoculation treatment were significant (Table 2). The only exception was the main effect for inoculation treatment for weight/100 dry seeds. Except for weight/100 seeds, all accession × inoculation interaction effects were also significant.

The 12 cowpea accessions exhibited a wide range of reactions to the *S. rolfsii*. Dry-seed yield/plant was severely impacted in several instances. For example, the dry-seed yields of Colossus 80, PI 354648, Climax, Magnolia Blackeye, Mississippi Silver, Mississippi Cream, and New Era were reduced by 25.1%, 26.3%, 16.8%, 49.4%, 27.6%, 28.3%, and 46.5%, respectively. Most of the cultigens with significantly impacted dry-seed yields also exhibited a significant increase in the wilt index. Alternatively, the dry seed yields of several accessions, i.e., Pinkeye Purple Hull, Brown Crowder, Carolina Cream, Speckled Purple Hull and Mississippi Purple, were not significantly impacted by the *S. rolfsii* inoculations, and only one of these cultivars exhibited a significant increase in the wilt index.

Examination of the yield components, number of pods/plant, number of seeds/pod, and weight/100 seeds suggests that most of the dry-seed yield losses caused by *S. rolfsii* can be attributed to reductions in the number of pods/plant. For example, the 49.4% reduction in the dry-seed yield/plant for Magnolia Blackeye was accompanied by a 45.7% reduction in the number of pods/plant and the 46.5% reduction in dry-seed yield/plant for New Era was accompanied by a 47.9% reduction in the number of pods/plant. It should be noted that there were several instances in which *S. rolfsii* did have some adverse impact on the number of seeds/pod and the weight/100 seeds, but these trends were not consistent across all accessions.

### 2.3. Re-evaluation of selected cowpea cultigens (1986 study)

Based on the development of stem lesions, this study confirmed the findings of the 1985 cowpea germplasm study indicating that the cultivars Brown Crowder and Carolina Cream are resistant to southern blight and that the cultivar Magnolia Blackeye is quite susceptible (Table 3). The main effects for the inoculation treatment were not significant for any of the measured variables, and there were no significant inoculation × accession interactions. This is probably because the uninoculated subplots of the susceptible cultivar Magnolia Blackeye exhibited as severe stem-lesion development in the inoculated subplots. Obviously, there was a sufficient amount of natural *S. rolfsii* inoculum in the soil to effectively inoculate virtually all the plants in the uninoculated control subplots. The stem-lesion development in the Magnolia Blackeye control subplots caused by natural infections seriously compromised the dry-seed yields.

## 3. Conclusions

The results of the studies reported here support the long-standing theory that southern blight is an important disease of cowpea. However, our data demonstrates

Table 3
Influence of southern blight on number of pods/plant, number of seeds/pod, weight/100 seeds, dry seed yield/plant, wilt index, and stem-lesion index of 'Brown Crowder', 'Carolina cream', and 'Magnolia Blackeye' (1986 study)

| Accession/inoculation treatment | Pods/plant (no.) | Seeds/pod (no.) | Wt. /100 dry seeds (g) | Dry-seed yield (g/plant) | Wilt index[a] | Stem-lesion index[b] |
|---|---|---|---|---|---|---|
| *Brown Crowder* | | | | | | |
| Uninoculated | 36.6 | 7.6 | 14.5 | 40.8 | 1.1 | 1.4 |
| Inoculated[c] | 38.9 | 7.7 | 14.6 | 44.4 | 1.2 | 1.7 |
| LSD (0.05)[d] | NS | NS | NS | NS | NS | NS |
| *Carolina Cream* | | | | | | |
| Uninoculated | 64.4 | 9.5 | 11.0 | 67.0 | 1.3 | 1.8 |
| Inoculated | 60.7 | 9.6 | 11.6 | 67.5 | 1.1 | 1.8 |
| LSD (0.05) | NS | NS | NS | NS | NS | NS |
| *Magnolia Blackeye* | | | | | | |
| Uninoculated | 59.3 | 7.2 | 14.4 | 61.9 | 1.8 | 3.4 |
| Inoculated | 63.7 | 7.4 | 14.2 | 66.6 | 1.2 | 3.6 |
| LSD (0.05) | NS | NS | NS | NS | NS | NS |
| | | | *F ratio values for main effects and interaction effects* | | | |
| Acession (ACC) | 18.09*** | 38.91*** | 131.09*** | 8.39** | 5.16* | 81.12*** |
| Inoculation (INOC) | 0.09NS | 0.67NS | 1.00NS | 0.64NS | 4.47NS | 1.86NS |
| INOC × ACC | 0.58NS | 0.01NS | 2.75NS | 0.11NS | 2.78NS | 0.21NS |

[a] Each plant was rated on a scale of 1–5; 1—no wilting symptoms, 2—slight or partial wilting, 3—general plant wilting, 4—permanent wilt, and 5—dead plant.

[b] Each plant was rated on a scale of 1–5; 1—no stem lesion, 2—small stem lesion ($\leqslant$25% of the stem circumference), 3—moderate stem lesion (26–50% of the stem circumference), 4—large stem lesion ($\geqslant$51% of the stem circumference), and 5—dead plant (stem completely girdled).

[c] Each plant in the inoculated plots was inoculated 31 d after seeding (inoculum level: 20 sclerotia/plant).

[d] Significance level between the inoculation treatments within the same cultivar treatment determined by calculation of LSDs for interaction effects. NS—Nonsignificant.

*Significant at $P \leqslant 0.05$.

**Significant at $P \leqslant 0.01$.

***Significant at $P \leqslant 0.001$.

that the impact of southern blight on cowpea seed yields can be considerably greater than that suggested by a review of the published literature. Reduced pod numbers likely accounted for most of the reduced seed yields. There is significant variability in the resistance of cowpea germplasm to southern blight. Two cultivars, Brown Crowder and Carolina Cream, exhibited promising levels of resistance in two years of testing.

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